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1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
4. TITLE AND SUBTITLE Laboratory transmission of Venezuelan equine encephalomyelitis virus by the tick <i>Hyalomma truncatum</i>		5. FUNDING NUMBERS
6. AUTHOR(S) Kenneth J. Linthicum and Thomas M. Logan		
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Walter Reed Army Institute of Research Washington, DC 20307-5100		8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research & Development Command Ft. Detrick, Frederick, MD 21702-5012		10. SPONSORING/MONITORING AGENCY REPORT NUMBER
11. SUPPLEMENTARY NOTES		
12a. DISTRIBUTION/AVAILABILITY STATEMENT APPROVED FOR PUBLIC RELEASE: DISTRIBUTION UNLIMITED		12b. DISTRIBUTION CODE
13. ABSTRACT (Maximum 200 words) To assess the vector potential of <i>H. truncatum</i> ticks for Venezuelan equine encephalitis (VEE) virus, larval ticks were allowed to feed on a viremic guinea pig infected with an epizootic virus while feeding on a viremic guinea pig, transstadially transmitted the virus to subsequent nymphs and adults, and transmitted the virus to susceptible hosts.		
14. SUBJECT TERMS Alphavirus, Togaviridae, Venezuelan equine encephalomyelitis, tick, <i>H. truncatum</i> , Acari, Ixodae		15. NUMBER OF PAGES
17. SECURITY CLASSIFICATION OF REPORT		16. PRICE CODE
18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT

NSN 7540-01-280-5500

ANNEX D

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18
298-102

Short Report

Laboratory transmission of Venezuelan equine encephalomyelitis virus by the tick *Hyalomma truncatum**

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Epizootic strains of Venezuelan equine encephalomyelitis VEE virus (*Alphavirus*, family *Togaviridae*) cause serious disease in horses and humans throughout the 'New World' tropics and subtropics (WALTON & GRAYSON, 1989). Although various mosquito species serve as vectors of this virus during epizootics, recent experimental evidence has indicated that ticks may be involved in the maintenance cycle (LINTHICUM *et al.*, 1992). With the rapid expansion of air travel between the Americas and Africa and Europe the potential for importation of VEE virus into the 'Old World' poses a threat to immunologically naive human and equine populations. *Hyalomma truncatum* Koch is a tick species commonly found in Africa and south-west Asia, and is a known vector of Crimean-Congo haemorrhagic fever virus (*Nairovirus*, family *Bunyaviridae*) (LOGAN *et al.*, 1989). To assess the vector potential of *H. truncatum* for VEE virus, larval ticks were allowed to feed on a viraemic guinea-pig infected with an epizootic VEE virus strain. Subsequently, ticks were evaluated to determine if virus replication occurred and if virus was transmitted.

H. truncatum ticks used in this study were maintained as described by LINTHICUM *et al.* (1991). All experiments were conducted in a B1.3+ laboratory specifically modified to contain ticks. Guinea-pigs used in this study had not been previously exposed to either VEE virus or ticks. The strain of VEE virus used (Trinidad donkey, variant 1-A) is almost always fatal to guinea-pigs. Virus content of sampled ticks was determined by plaque assay on Vero cell monolayers (LINTHICUM *et al.*, 1992).

Initially one guinea-pig was infested with approximately 2000 tick larvae. One day later, the guinea-pig was inoculated subcutaneously with $10^{6.2}$ plaque-forming units (PFU) of VEE virus. On day 4 after infestation the serum viral titre in the guinea-pig was $10^{5.5}$ PFU mL. The guinea-pig died 5 d after infestation.

More than 800 fed larvae dropped off the guinea-pig 4–5 d after infestation. All 10 fed larvae sampled after dropping off contained VEE virus (mean titre = $10^{2.6}$ PFU, range $10^{1.6}$ – $10^{3.3}$). Fed larvae started to moult 12 d after infestation, and at 14 d after infestation 3 of 21 pools of unfed nymphs [minimum infection rate = 3/105 (2.9%)] contained virus (mean titre = $10^{4.7}$ PFU, range

$10^{4.0}$ – $10^{5.7}$). On day 14 after infestation about 100 of these unfed nymphs were placed on another guinea-pig, which died 6 d later; however, no virus was isolated from it or from 45 fed nymphs 2 d after they had dropped off the animal.

On day 21 after infestation of the first guinea-pig, none of 95 unfed nymphs sampled contained virus; however, when 100 unfed nymphs were placed on a guinea-pig the animal died 6 d later with a serum viral titre of $10^{7.1}$ PFU mL. At drop-off, 6 of 7 (86%) fed nymphs contained virus (mean titre = $10^{3.6}$ PFU, range $10^{2.8}$ – $10^{4.3}$). About 80 partially fed nymphs were transferred to another guinea-pig, which died 4 d later, with a serum viral titre of $10^{6.1}$ PFU mL, and 28/31 (90%) fed nymphs contained virus (mean titre = $10^{4.3}$ PFU, range $10^{1.9}$ – $10^{5.3}$) when they dropped off. At 56 days after infestation 3 of 14 (21.4%) subsequent adults contained virus (mean titre = $10^{3.9}$ PFU, range $10^{2.5}$ – $10^{4.6}$).

On day 28 after infestation, 2 of 40 pools of unfed nymphs [minimum infection rate = 2/200 (1%)] contained virus (mean titre = $10^{2.1}$ PFU). About 200 unfed nymphs were placed on a guinea-pig at this time, and the guinea-pig survived. Only 1 of 124 (0.8%) fed nymphs contained virus (titre = $10^{5.0}$ PFU) when they dropped off.

The ability of *H. truncatum* larvae to become infected with VEE virus while feeding on a viraemic guinea-pig, transstadially transmit the virus to subsequent nymphs and adults, and transmit the virus to susceptible hosts, indicates that this species is a competent laboratory vector of the virus. Infection and transmission rates, and the viral titres observed for *H. truncatum*, are equal to or greater than those observed previously for *Amblyomma cajennense* infected with the same strain of virus (LINTHICUM *et al.*, 1992). Thus if VEE virus were introduced into south-west Asia or Africa it could be maintained in, and transmitted by, an indigenous tick species.

Acknowledgements

We thank J. Kondig for his invaluable professional and technical contributions to this study and R. E. Coleman, S. Frances, K. Kenyon, R. Rosenberg, and M. J. Turell for their critical review of the manuscript. Research was conducted in compliance with the Animal Welfare Act and other US Federal statutes and regulations relating to animals and experiments involving animals and adhered to the *Guide For the Use of Laboratory Animals*, NIH Publication 86–23, 1985 edition. The views of the authors do not necessarily reflect the position of the Department of the Army or the Department of Defense.

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Received 14 May 1993; accepted for publication 23 June 1993

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Received 28 October 1992; revised 5 April 1993; accepted for publication 13 May 1993

Accession For	
NTIS CRA&I	✓
DIIC TAB	✓
Univ. of Calif	✓
Justification	
By	
Distribution /	
Availability Codes	
Dist	Avail. and/or Special
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Corrections

F. Pratlong et al. 1993. Characterization of *Leishmania* isolates from two AIDS patients originating from Valencia, Spain. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **87**, 705-706.

The international code numbers of 2 of the strains of *Leishmania infantum* isolated from these patients were incorrectly printed on p. 705: 3 lines from the bottom of column 2; and p. 706: line 13 of column 1; the correct numbers are MHOM ES 91 LEM2298 and MHOM ES 91 LEM2361, respectively. The editor apologizes for these errors.

M. Corcos and C. Corcos 1993. A transposon in Hansen's bacillus? [Correspondence]. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **87**, 708.

The authors have pointed out that the word 'its', in line 4 of paragraph 5 of their letter, appeared as 'whose' in the original typescript, and that this more clearly indicates their meaning, that it is the replication of the *plasmid* which is an epiphenomenal self-perpetuating feedback process.